#17 YC 10-24-02

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants:	Groman et al.	Atty Docket:	1275/190
Serial No.:	09/521,264	Art Unit:	1619
Date Filed:	March 9, 2000	Examiner:	Wells, L.
Invention:	Heat Stable Colloidal Iron Oxides Coated with Reduced Carbohydrates and Carbohydrate Derivatives		

Commissioner for Patents
Washington, DC 20231

DECLARATION OF JEROME M. LEWIS, Ph.D. IN SUPPORT OF APPLICANTS' RESPONSE [37 C.F.R. § 1.132]

Dear Sir:

In further support of the response to the Office Action mailed November 2, 2001, in the above-reference matter, and in response to the Office Action mailed August 12, 2002, I hereby declare as follows:

1. My name is Jerome M. Lewis, Ph.D. I am Vice President of Scientific Operations, of Advanced Magnetics, Inc., the assignee herein, and one of the inventors of the subject matter of the above patent application. I am an inventor or co-inventor of a substantial number of patents involving (among other things) polysaccharide superparamagnetic iron oxide complexes and related materials and methods. I am also familiar with and have supervisory responsibility at Advanced Magnetics, Inc. in carrying out Current Good Manufacturing Practices (cGMPs) as required by the Food and Drug Administration. My

further credentials are set forth in my Curriculum Vitae, which is attached as Exhibit A with my Declaration submitted with Response B on February 28, 2002.

2. I have read the action of August 12, 2002. This declaration is provided to clarify the record concerning polysaccharide superparamagnetic iron oxide complexes, and in particular to set forth the distinguishing differences between the technology and compounds disclosed in the Maruno patent (the '378 patent) and those disclosed and claimed in the present application.

Consideration of the Prior Art

3. As stated in my original Declaration, it would be highly desirable, from both regulatory and commercial perspectives, to have an ultrasmall polysaccharide superparamagnetic iron oxide complex as a pharmaceutical that when autoclaved i) does not form particulates and ii) causes minimal edematous response in a subject upon injection into the subject. I show below with experimental data that, until the present invention, no such material existed and it would not have been obvious to prepare such a material, based on the prior art and technology known at the time our invention was conceived and reduced to practice.

4. First, with respect to the cited Maruno patent, that '378 patent discloses carboxyalkyl-ether polysaccharide derivatives for use as NMR imaging contrast agents. For example, compound 3 (see Table 3, col. 17), is prepared by a synthetic process involving heating at reflux, centrifugation to remove particulates, precipitation with methanol, and finally filtration through a 0.45 μm filter (see col. 17, Example 2 and Table 3, lines 20-55).

5. Compound 3 of the '378 patent, compared to other compounds disclosed in this patent, is the compound most similar to our compound 31, with respect to extent of carboxymethylation and molecular weight of the carbohydrate (see the '378 patent, Table 1), and which also, based on percent yield, particle size, and stability, is the best compound disclosed in the '378 patent (see Table 3).

6. We independently prepared compound 3 ("the Maruno compound") of the '378 patent according to the method disclosed in the '378 patent. We determined that the synthesis procedure disclosed in the '378 patent for this compound yields a wide range of particle sizes, from very small to very large as to be expected from any synthesis which uses centrifugation to remove larger particles. Indeed, we found that in this procedure it is the only the final centrifugation and filtration steps (described in Maruno '378) which serve to limit the upper range of particle size to ~ 0.5 μm, not the synthesis itself.

7. We also observed that the Maruno compound begins to precipitate even during synthesis - thus the disclosed centrifugation step after reflux. Our experiments indicate that the original full range of products prepared by this synthetic route contain a significant level of products which immediately precipitate during, and continue accumulating after, the reflux step. Without centrifugation after reflux, it is not possible to filter sterilize because the high level of precipitated product clogs the filter. As determined experimentally in our laboratory, upon centrifugation, a sizable pellet of solid product is removed at this stage.

8. Nevertheless, even with centrifugation after reflux, a significant amount of larger material is present. Filtration through a 0.45 μm membrane filter removes these

additional precipitates, and also simultaneously serves to reduce the upper limit of particle size to ~ 0.5 μm.

9. Third, it is impossible to prepare the Maruno compound in the size range he claims (i.e. those with a particle size between 0.01 μm and 0.5 μm - see claim 13) without a filtration step, or even without a centrifugation step, because precipitation occurs during synthesis during the reflux step, and continues with time, even after filter sterilization and concomitant particle size reduction.

10. Finally, our laboratory subjected the synthesized Maruno compound to temperatures of both 80 °C and 121 °C (autoclaving), and compared the results to the same tests performed on various products available in our laboratory, including that prepared according to Example 31 in the present application. **Table 1** below summarizes the results. As can be clearly seen, after treating at 121 °C for 30 min., there is an order of magnitude more particulates observed in the Maruno compound, whether particulates >10 μm or >25 μm, than with our Example 31, even at 1.3 mm COOH/g CMRD. Further, there is an order of magnitude more particulates in the Maruno compound at 121 °C compared to particulates in the Maruno material at room temperature (untreated).

Table i

	Untreated particulates/mL		80 °C for 30 min. particulates/mL		121 °C for 30 min. particulates/mL	
	>10μm	>25μm	>10μm	>25μm	>10μm	>25μm
CMRD-USPIO, 0.37 mM COOH/g CMRD	2	1	4	1	398	287*
CMRD -USPIO, 0.58 mM COOH/g CMRD	1	2	1	1	381	269*
CMD-USPIO, Example 30 1.2 mM COOH/g CMD	0	0	0	0	706	432*
Combidex , dextran T-10	0	0	0	0	>1000	>1000*
Code 5127 (Combidex precursor) dextran T-10	7	4	12	8	congealed	congealed *
Maruno's Compound 3, 1.3 mM COOH/g CMRD	6	4	10	6	60	51
CMRD -USPIO, Example 31, 1.3 mM COOH/g CMRD	4	1	-	-	7	5

* Contained particles very much larger than 25 μm.
CMRD -USPIO: reduced polysaccharide carboxymethylated ultrasmall superparamagnetic iron oxide
CMD -USPIO: reduced polysaccharide carboxymethylated ultrasmall superparamagnetic iron oxide
COOH: carboxyl group

11. In contrast, the presently claimed material (see in particular Example 31, p. 36 of the Application) can be prepared without any filtration or centrifugation step at all. Our disclosed synthetic method produces a very narrow size range of ultrasmall particles having the desired particle size from the outset without any filtration or need to limit particle size below a specific diameter. Our synthetic method does not require a reflux step or centrifugation. And the resulting material does not fall apart upon autoclaving – i.e., heating at 121 °C for 30 min. does not result in an increase in particulate formation.

12. In addition, current experimental data indicate that such autoclaved materials are stable (as measured by no increase in particulate level per mL of material) for up to 3 years. Based on the very different and specific synthetic parameters disclosed in our

application (see specification, Examples 31-36, pp. 36-39, and Table ii, below), and the very different resulting properties (narrow range of molecular weights having the desired particle size without centrifugation and filtration, extended stability without increase in particulate formation, and capacity to be autoclaved without degradation, as evidenced by particulate formation) our claimed carboxyalkylated reduced polysaccharides are completely different compounds from those disclosed and claimed in the Maruno patent.

lot	notes	[Fe], mg/g	Fe(III)/Fe(II), Mmol	base/Cl, mmol	PS/Fe, g/g
7228	base=28% NH_3	6.42	1.472	3.90	3.85
Maruno compound 3	base=3 M NaOH	15-19	1.541	1-2	5.55

PS = polysaccharide
Drug substance of 7228 = Example 31

13. The present application is directed to the third generation of material developed by Advanced Magnetics, and this material is presently in clinical trials. This material, like Combidex, is a complex of ultrasmall particles and has a similar favorable biodistribution. This material is terminally sterilized, and unlike Feridex and Combidex, has a sufficiently small risk of particulate formation such that no filtration by the physician is required during administration.

14. In summary, it would not be obvious for one of ordinary skill in the art to replace the filtration step of Maruno with autoclaving because first, material in Maruno cannot be made at all without the centrifugation and filtration steps, and second, Maruno material degrades substantially at 121 °C after 30 minutes, and continues to degrade after filtration, as evidenced by increased levels of particulates with time.

15. Lastly, the analysis comparing previous-generation Advanced Magnetic compounds Feridex and Combidex (disclosed in the Lewis and Groman patents cited by the Examiner to support the obviousness rejection) with the new material of the presently claimed invention is summarized in the following table, wherein comparison with Maruno's compound 3 material is also now included:

Material	Sterilization method	Filtration during administration required to reduce risk of particulate formation?	Dilution and slow administration required to reduce adverse reaction?	Edematous response?	Possible to synthesize without filtration & centrifugation?
Feridex (Lewis, 5,055,288; Groman, 4,827,945)	Autoclaving possible only following citrate addition	Yes	Yes	Yes	No
Combidex (Josephson, 5,160,726)	Filtration	Yes	Yes	Yes	Filtration required
Maruno #3	Filtration	Yes	Not known	Not known	No
New material (in present application)	Autoclaving	No	No	No	Yes

16. The materials of Maruno do not meet the requirements of the materials in the presently claimed invention, and furthermore, are inconsistent with the desirable regulatory, commercial and medical properties of a contrast agent. There is no teaching in Maruno of how to make stable materials that survive autoclaving. Nor is there any teaching in Maruno of materials that have decreased adverse reactions (measured in laboratory animal experiments as edematous response).

17. As stated in my original Declaration, the materials of Maruno are filter sterilized. Based on our experimental results with the disclosed Maruno synthesis and materials, the

only reason for using filter sterilization instead of terminal sterilization is the instability of the Maruno material at temperatures required for autoclaving, as alluded to by Maruno himself in the '457 patent ("similar tendency [i.e. deposition of precipitate or gelation] is observed in an autoclave at 110 °C to 130 °C," see col, 11 lines27- 31). Our experiments with Maruno compound 3, along with Maruno's absence of testing data above 80 °C and Maruno's continued use of filter sterilization in the later '378 patent (filed nearly 8 years after the '457 Maruno patent), confirms that Maruno '378 material is indeed subject to precipitation or gelation at higher (i.e. autoclave) temperatures. In fact, most of the examples in Maruno '457 – all but 3 and 7 – suffer the same fate, even at the lower temperature of 80 °C. And Maruno '378, despite having been filed over 7 years later, contains no testing data whatsoever for stability at elevated temperatures. In short, the Maruno references teach that the materials disclosed therein suffer instability when subjected to temperatures above 80 degrees C, and our experimental results confirm this.

18. In conclusion, the Maruno references fail to teach anything about creating a material that can be autoclaved without degradation. Finally, the new material (the subject of the present application) does not require filtration during synthesis to achieve the desired particle size, is stable to autoclaving, does not require dilution for administration, and does not require filtration during administration, none of which criteria are satisfied by the Maruno materials.

19. I hereby declare that all statements made herein are of my own knowledge and that all statements made on information and belief are true; and further that these statements are being made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both under Section 1001 of Title 18 of

the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Jerome M. Lewis, Ph.D.

Dated: September 25, 2002

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